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Method and Apparatus for Performing Microassays

The present application claims priority from U.S. provisional application number
5 60/071,179 filed January 12, 1998, which application is herein incorporated by reference.

Technical Field

The present invention pertains to an apparatus and method for manipulating,
transporting, and analyzing a large number of microscopic samples of a liquid or of materials
10 including cells currently or formerly in liquid suspension.

Background of the Invention

Chemistry on the micro-scale, involving the reaction and subsequent analysis of
quantities of reagents or analytes of order microliters or smaller, is an increasingly important
15 aspect of the development of new substances in the pharmaceutical and other industries. Such
reaction and analysis may accommodate vast libraries containing as many as a million
compounds to be reacted and analyzed under various conditions. Significant problems
associated with current technologies as applied to chemical analysis of vast numbers
(potentially on the order of hundreds of thousands or millions per day) of compounds include
20 the problem of handling vast numbers of compounds and reactions in parallel.

Existing technology relies on 96-, 384-, or 1536-well plates containing quantities
between approximately 1 microliter and 1 milliliter of liquid compound per well, and,
generally, involves chemical reactions and analysis in wells disposed with single openings on
flat, two-dimensional surfaces such as silicon chips. It is not practical to apply existing
25 technology in the art to form million-well disks. There is a need, therefore, for new
approaches that permit the analysis of a million samples in a laboratory format.

Summary of the Invention

In accordance with one aspect of the invention, in one of its embodiments, there is
30 provided a method for selecting samples having specified properties from a library of
samples. The method has the steps of:

- a. providing a platen having two substantially parallel planar surfaces and a

plurality of addressable through-holes disposed substantially perpendicularly to the planar surfaces;

- b. loading a first sample in liquid form into at least one of the through-holes;
- c. adding a second sample into the at least one of the through-holes for
- 5 permitting a reaction between the first sample and the second sample; and
- d. characterizing the reaction in the through-hole in terms of the specified properties.

In accordance with alternate embodiments of the invention, each through-hole may be dimensioned so as to maintain a liquid sample therein by means of surface tension, and may
10 have a volume less than 100 nanoliters. The plurality of addressable through-holes may have a density in excess of 10^8 per square meter.

In accordance with further alternate embodiments of the invention, the step of loading a first sample may include drawing the sample from a planar surface by capillary action. The platen may be brought into contact with a reservoir of liquid and rotated about an axis
15 perpendicular to the surface of the reservoir or about at least one of an axis perpendicular to the surface of the reservoir and an axis parallel to the surface of the reservoir. The method may include the further step of maintaining a humid atmosphere for preventing evaporation of the first sample or coating the liquid sample with a monolayer for preventing evaporation of the first sample.

20 In accordance with a further aspect of the present invention, a method is provided for preparing a plurality of combinations of members of a first set of samples in liquid form with members of a second set of samples in liquid form, the method comprising:

- a. providing a first perforated platen having through-holes and a second perforated platen having through-holes;
- 25 b. loading a first set of samples in liquid form into the through-holes of the first perforated platen;
- c. loading a second set of samples in liquid form into the through-holes of the second perforated platen;
- d. registering the through-holes of the first perforated platen with the through-holes of
30 the second perforated platen; and
- e. combining the first set of samples with the second set of samples.

In accordance with yet further aspects of the present invention, there are provided

methods for mixing and diluting liquid samples. The methods have steps of loading one set of liquid samples into through-holes of a first platen and loading another set of liquid samples into through-holes of a second platen, and then disposing a surface of the first platen in contact with a surface of the second platen in such a way as to register at least one through-hole of the first platen with at least one of through-hole of the second platen for permitting mixing of the liquid samples of the respective sets.

In accordance with another aspect of the present invention, there is provided a system for analyzing a plurality of liquid samples. The system has a platen having two substantially parallel planar surfaces and a plurality of through-holes having apertures and walls, a source of optical radiation for illuminating at least one through-hole along an optical axis, and an optical arrangement for analyzing light emanating from the at least one through-hole.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference to the following detailed description taken with the accompanying drawings in which:

FIG. 1 is a side view in cross-section of a portion of a laminated platen containing multiple through-holes for analysis of liquid samples in accordance with a preferred embodiment of the present invention;

FIG. 2A is top view of a portion of the platen of FIG. 1 in which the through-holes are configured on rectangular centers;

FIG. 2B is top view of a portion of the platen of FIG. 1 in which the through-holes are configured in a hexagonal close-packed array;

FIG. 3 is a top view of round sample wafer populated with through-holes in accordance with an embodiment of the present invention;

FIG. 4 is a side perspective view of an arrangement for loading a liquid sample into the platen of FIG. 1 by employing capillary and inertial insertion forces;

FIG. 5 is a cut-away view of a single through-hole in the platen of FIG. 1, showing the use of hydrophobic and hydrophilic layers for containment of an aqueous sample;

FIG. 6 is schematic diagram of a confocal optical arrangement for interrogation of a liquid sample in a through-hole in accordance with an embodiment of the present invention;

FIG. 7 is perspective view of a scanning arrangement for serially interrogating liquid samples retained in through-holes of a disk-type platen in accordance with an embodiment of

the present invention;

FIG. 8 is schematic representation of a scanning arrangement for serially interrogating liquid samples retained in a continuous-process film-type platen, in accordance with an alternate embodiment of the present invention;

5 FIG. 9 is a cross-sectional view of portions of two platens brought into proximity with through-hole registration in anticipation of mixing or dilution in accordance with embodiments of the present invention; and

FIG. 10 is a cross-sectional view of the portions of two platens of FIG. 9 after the two platens have been brought into contact to facilitate mixing or dilution.

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Detailed Description of Preferred Embodiments

Through-hole wells

In accordance with a preferred embodiment of the invention, the volume of each well employed for the assay of a chemical or biochemical reaction is reduced typically to less than 15 100 nanoliters (10^{-10} m^3). The packing density of wells may thereby be increased by several orders of magnitude over prior art technology. Referring to FIG. 1, a side view is shown in cross section of a platen 10, otherwise referred to herein as a "substrate" or "sample wafer." Platen 10 is the carrier of a large number of through-holes 12 which traverse platen 10 from one surface 14 to an opposing surface 16 of the platen and constitute assay wells (or 20 "microwells") in accordance with an embodiment of the invention. Through-holes 12 may be shaped as circular right cylinders, or, alternatively, may have rectangular cross-sections, however otherwise shaped through-holes are within the scope of the present invention. As used in the present description and in the appended claims, the term "platen" refers to a structure having substantially parallel plane surfaces and transverse dimensions substantially 25 exceeding the thickness of the structure between the substantially parallel plane surfaces.

The apertures of through-holes 12 need not be square, and, in accordance with an alternate embodiment of the present invention, flanges 8 may extend above planar surface 14 surrounding some or all of through-holes 12 while indentations 6 may be fabricated rounding the edges of through-holes 12 at opposing surface 16. Flanges 8 and indentations 6 may 30 advantageously provide for registration of successive platens 10, in the case where platens are stacked, and in processes of mixing or dilution, as discussed in detail below in reference to

Figs. 9-10.

In accordance with an embodiment of the invention, through-holes 12 are loaded with a first sample 18 in liquid form. Sample 18 is allowed to react with a second sample where the second sample may include a variety of test samples and by subsequent or concurrent
5 analysis of the reaction products, using, for example, optical markers, a large number of reactions may be processed and analyzed in parallel.

As applied to biological assays, by way of example, first sample 18 may be a reagent, including, for example, cells in aqueous suspension, eukaryotic (animal, yeast) or prokaryotic (bacteria) cells, hybrid cells, and biological molecules including, for example, antibodies and
10 enzymes, although application to other biological or non-biological assays is within the scope of the invention as claimed herein. All such reagents may also be referred to herein and in the appended claims as "targets." Typical yeast cell concentrations of 10^7 cells per milliliter of solution yield on the order of 1000 cells per 100 nanoliter well. Typically, an entire chip or the subset of through-hole wells constituting a contiguous region of platen 10 may be
15 populated with a single strain of cells.

A typical procedure assay procedure, such as may be employed in pharmaceutical research, entails the subsequent addressed introduction of a test sample including one or more analytes into the through-hole wells, with selected materials introduced into subsets of through-holes that may include one or more through-holes. The test sample addressably
20 introduced into the subsets of through-holes may contain drug candidates or known drugs. The test sample may be comprised of multiple components, introduced at the same time or sequentially. Components of the test sample may include analytes, antagonists, reagents, solvents, or any other materials and may be introduced in liquid form or otherwise. In accordance with a preferred embodiment of the invention, test samples are introduced into the
25 through-hole wells in liquid form in order to facilitate rapid reaction via diffusion with first sample 18 already resident in liquid form in the through-holes.

The set of substances from which the second sample addressed to a particular through-hole site is drawn is referred to in this description and in the appended claims as a "library" of substances. In typical applications, the library is of a substantial size and thus
30 advantageously utilizes the capability of the present invention to facilitate parallel reaction and analysis of large numbers of substances. In pharmaceutical applications in particular,

libraries may be composed of between 10^3 and 10^9 substances and combinations of substances.

A typical thickness **20** of platen **10** is on the order of 1-2 mm, while through-holes **12** have typical characteristic dimensions (such as diameters) **22** of on the order of 100-400 μm .

5 Thus the volume of each through-hole **12** between surface **14** and surface **16** is on the order of $\sim 10^{-7} \text{ cm}^3$ or greater. Through-holes **12** are spaced on centers typically on the order of twice the diameter of the holes, although all spacing configurations are within the scope of the invention and of the appended claims. In particular, through-holes **12** may be centered on a rectangular grid, as shown in FIG. 2A, or in a close-packed hexagonal lattice, as shown in
10 FIG. 2B.

In accordance with an alternate embodiment of the present invention described with reference to FIG. 3, through-holes **12** may be disposed in an array within a circular sample wafer **300** having a central hole **302** for purposes of centering with respect to handling equipment.

15 Referring again to FIG. 1, platen **10** may be any solid or quasi-solid material into which through-holes **12** may be formed. In particular, in accordance with various embodiments of the invention, platen **10** may be formed from metal, semiconductor, glass, quartz, ceramic or polymer materials, all given without limitation by way of example. In accordance with a preferred embodiment of the invention, platen **10** is formed in a format
20 associated with a compact disk read-only-memory (CD-ROM) - namely that of a polymer disk, approximately 1.2 mm in thickness, and approximately 100 mm in diameter.

Platen **10** may also advantageously be formed of a laminate of materials, with a central layer **26** and outer "sandwiching" layers **28**. Advantages of this construction for containment of sample **18** will be discussed further below.

25 Through-holes **12** may be formed in platen **10** by means appropriate to the material of platen **10**. Through-hole forming methods include, by way of example, laser ablation by means of an ultraviolet (UV) excimer laser which may form 100 μm through-holes in glasses and polymers. Additional through-hole forming techniques include mechanical drilling, electrochemical methods, or selective chemical or charged-particle etching techniques.
30 Additionally, microcapillary bundles of glass fibers of varying compositions may be drawn from preform and sliced to form platens, and then selectively etched to form through-holes.

Loading the through-hole microwells

On the size scale employed in accordance with embodiments of the invention, where through-holes **12** have aspect ratios of axial length to diameter greater than unity, viscous forces may dominate inertial forces in governing the fluid kinetics of material in the through-hole wells. Consequently, capillary action may be employed to populate through-holes **12** with sample fluid **18**. Referring to FIG. 4, two aspects of loading the through-hole wells are described with reference to a sample insertion apparatus **30**. Since through-hole microwells **12** are open at both sides, insertion of liquid into the wells does not require that the air displaced by the liquid on insertion flow through the entering fluid, as occurs in the prior art well structure having only a single aperture for influx of liquid and efflux of displaced air. Liquid **32**, loaded into reservoir **34** via port **33**, may, as discussed above, contain cells or other particles in suspension. Liquid **32** may be forced into through-hole microwells **12** (shown in FIG. 1) by in-line impulsion as by driving platen **10** into liquid **32** by force applied along direction **36** transverse to the plane of platen **10**. The transverse piston force may be applied via shaft **38** or in any other manner known in the mechanical arts.

In accordance with another embodiment of the invention, liquid may also be loaded through capillary action of liquid **32** along the walls of the through-holes. To provide for wetting of the lower surface of platen **10**, the platen is lowered into reservoir **34** and rotated, by torque applied through shaft **38**, or otherwise, through an angle typically on the order of a quarter revolution. Alternatively, platen **10** may be wetted and liquid **32** drawn into the microwells by immersing platen **10** into liquid **32** and tilting the platen about an axis in the plane of the platen.

Stabilization with respect to capillary and evaporative liquid loss

In order to maintain the sample in liquid form in the respective microwells, evaporation of the liquid must be avoided. One method of avoiding evaporation is to provide an ambient atmospheric environment of 100% humidity. Among other methods that may be practiced to suppress evaporation, in accordance with an embodiment of the invention, a high molecular-weight fluid, such as various alcohols, for example, may be introduced on each end of the microwells thereby forming molecular monolayers or other thin layers to prevent evaporation of the liquid sample.

Referring to FIG. 5, a cross-section of a portion of platen **10** is shown to include through-hole microwell **12**. In order to enhance capillary loading of the microwell and to prevent capillary outmigration of the sample liquid, exterior sections **40** of the microwell, adjacent to surfaces **14** and **16** of platen **10**, has a hydrophobic wall surface in accordance with a preferred embodiment of the invention, while the interior section **42** of the through-hole wall has a hydrophilic surface thereby preferentially attracting an aqueous liquid sample. Typically, the interior $\sim 160\ \mu\text{m}$ segment of the microwell may have a hydrophilic wall surface, while the hydrophobic layers on either end of the well are on the order of $20\ \mu\text{m}$ in length. On loading the sample liquid into the microwells, typically 10% of the well, on either end, is left unfilled, and subsequent test samples in liquid form will rapidly diffuse to hydrophilic center of microwell thereby mixing with the liquid already present.

Optical interrogation

Depending upon the application to which the present invention is applied, the result of the reaction of the first sample in liquid form with subsequently added analytes may be read out in a wide variety of manners known to persons skilled in the biological or biochemical arts. Readout systems may employ taggants of various sorts allowing interrogation of the sample within the addressable microwell to determine whether a specified reaction has occurred. Some reactions may be interrogated optically, to include, without limitation, such optical methods as colorimetric or fluorometric methods, or resonant or non-resonant scattering methods, including Raman spectroscopic methods.

Referring now to FIG. 6, optical interrogation methods, of which the foregoing are but examples, may be implemented, in accordance with an embodiment of the invention by coupling a light beam **50** into through-hole **12** of platen **10** and detecting light **52** emergent from the opposite aperture of through-hole **12** by detectors **54** constituting detector array **56**. Alternatively, light returned by scattering in the original direction can be collected and analyzed using standard optical techniques. In order to optimize the signal-to-noise of the optical signal, the beam shape and through-hole volume are preferably matched. In accordance with a preferred embodiment of the invention, optical matching to a through-hole of cylindrical cross-section and of aspect ratio greater than one is achieved through a confocal optical geometry in which an initially collimated beam **50** is transformed by optical element

58 into a beam having a diffraction limited focus at the center 60 of through-hole 12. The emergent optical beam 52 is collected and focussed onto detector array 56 by optical element 60. Superior optical sampling of the volume of the through-hole may be obtained if the through-hole has a rectangular cross-section, and if the optical radiation is guided by the walls of the through-hole in the manner of a waveguide. Optical element 58 and 60 may be lenses or mirrors or combinations thereof as well known to persons skilled in the optical arts. Detector array 56 may be a charge-coupled device (CCD) array, for example, and, in one embodiment of the invention, a 1000 × 1000 element format is employed, with each through-hole imaged onto three elements 54 of the detector array. A window 62 may be disposed between platen 10 and detector array 56 and may be dried using standard techniques if the assay is conducted in a humid ambient environment as discussed above. Alternatively, beam 50, coupled into through-hole 12 by coupling element 58 may be guided, in the manner of a guided wave through a waveguide, by the walls 62 of through-hole 12 in order to provide efficient interrogation of the sampled volume within the through-hole.

15 In some cases, where the material of platen 10 is not entirely opaque at the wavelengths of interrogating optical beam 50, wall 62 of through-hole 12 may be coated to prevent light leakage and cross-talk among the addressable sample volumes.

FIG. 7 shows a preferred embodiment of the present invention in which platen 10 is configured in the CD-ROM format described above, with interrogating optical source 50 capable of travel in radial direction 68 while platen 10 rotates about center 66. Optical detector array 56 may translate in conjunction with source 50, in accordance with an embodiment of the invention.

Continuous process analysis

25 Referring to FIG. 8, in accordance with an advantageous embodiment of the present invention, platen 10, which may be a flexible polymeric substance, for example, is conveyed in a direction 70 past an optical interrogation system comprising an optical source 72 and a detector array 74. Samples in liquid form may be loaded into through-holes 12 and advanced at a rate governed by the relevant reaction times so that a row 76 is interrogated optically at the period during which a specified indication is expected.

Mixing and Dilution

Referring now to Fig. 9, a cross-sectional view is shown of portions of a first platen **90** and a second platen **92** brought into proximity with each other in anticipation of processes performed in accordance with embodiments of the present invention for preparing, mixing, or diluting liquid samples. Through-holes **12** of platen **90** are shown as having been loaded with liquid samples **94** which may be identical across some specified subset of through-holes **12**, or may be identical for the entire platen. Liquid sample **94**, as shown schematically, may include cells or other targets **96** in solution within a solvent **98**.

Through-holes **12** of second platen **92** is shown as having been loaded with liquid samples **100** and **102** shown comprising one or more solvents or other agents. In particular, platen **92** may have been populated with a library of distinct compounds, each of which is to be exposed to target **96** of platen **90**.

Fig. 10 shows platens **90** and **92** of Fig. 9 having been brought into contact with one another, in such a manner as to allow through-holes of the respective platens to register on a one-to-one basis. The mating of protrusions **8** with indentations **6** of respective platens facilitates the registration of through-holes, and provides for the mixing of the liquid sample contents of the respective through-holes. Thus, as shown, half of targets **96** from samples **94** of first platen **90** have migrated to the solvent of samples **100** and **102**. Mixing or dilution may be facilitated in this manner, either through ordinary statistical diffusion, or by any method employed to facilitate mixing. Mixing may be enhanced, for example, by the creation of thermal eddy currents and turbulence induced by laser irradiation. Mixing rates have been found to be enhanced in this way by more than an order of magnitude. Any other mixing techniques, including acoustic perturbation or stirring of the samples with micropipettes, for example, are within the scope of the present invention as described herein and as claimed in any appended claims.

The number of platens **90** and **92** that may be stacked, in accordance with the present invention, is not limited to two, as shown in Figs. 9 and 10 by way of example only. Thus, the concentration of targets **96** in solvent **98** may be diluted to a specified degree by stacking a corresponding number of platens with registered through-holes and allowing migration of targets **96** throughout the liquid contained within the corresponding sample volumes of the stack.

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